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AUTISM SPECTRUM DISORDER AND OBSTETRIC OPTIMALITY: A TWIN STUDY AND META-ANALYSIS OF SIBLING STUDIES

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Abstract

Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder with a strong genetic basis. Recent studies have suggested that its aetiology is also influenced by environmental factors. Some of the most examined environmental factors are obstetric complications. However, the results are inconsistent.

Methods: We aimed to explore the association between obstetric complications and autism in a population-based twin sample using the Obstetric Enquiry Scale (OES), a scale that measures the presence or absence of pre-, peri-, and neonatal factors. Additionally, we report the meta-analytic results for obstetrical factors reported in previously published sibling studies.

Results: Our study included 115 cases pairs and 62 controls pairs and showed that children with autism and their unaffected co-twins present significantly more obstetric complications than controls (ASD vs controls β 1.26, CI 95% 1.11-1.40 $p < .001$; unaffected co-twin vs controls β 1.20, 95% CI 1.07-1.36 $p < .003$). However, we did not find statistically significant differences between children with ASD and their unaffected co-twins (β .96, 95% CI .85-1.09, p .55). Meta-analysis demonstrated that maternal hypertension (RR 1.35, CI 95% 1.23-1.48), uterine bleeding (RR 1.20 CI 95% 1.01-1.42) and exposure to antibiotic during pregnancy (1.11 CI 95% 1.00-1.22) increase risk of ASD.

Conclusions: This study confirms that children with ASD and their unaffected twins show more obstetric complications than controls. However, these complications do not distinguish between ASD twins and their unaffected co-twins. In addition, the meta-analysis showed little influence of birth factors on ASD which suggests a shared familial liability for both obstetric complications and autism, rather than a causal association.

Keywords: Autism Spectrum Disorder, obstetric complications, genetics.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder (NDD) characterized by deficits in social communication, and restricted and repetitive behaviours (American Psychiatric Association., 2013). The current estimated prevalence ranges from 1.31% to 3.14%, with an approximately 4:1 male:female ratio (Maenner, Shaw, & Baio, 2020)

The most recent studies suggest that autism is a heterogeneous condition, and its aetiology is complex and strongly influenced by genetic factors. The heritability of ASD has been reported to be over 80% as estimated from twin studies (Colvert et al., 2015; Sandin et al., 2017; Tick, Bolton, Happé, Rutter, & Rijdsdijk, 2016), with little influence of shared environment, that is environmental factors affecting both members of a twin pair (Tick et al., 2016). Nonshared environment (i.e., environmental influences affecting members of a twin pair differently) is estimated to explain 20% of individual differences in autistic traits (Colvert et al., 2015; Ronald et al., 2006).

In an attempt to find nonshared environmental factors that play a role in ASD, several authors have analysed obstetric factors. Studies using 'obstetric optimality' scores, to summarise the presence/absence of a range of adverse pre- and peri-natal factors, have shown mixed results in relation to ASD. Zwaigenbaum et al found that autistic children had lower obstetric optimality scores than their nonautistic siblings (Zwaigenbaum, Szatmari, & Jones, 2002). However, Lord et al concluded from their data that the association between ASD and obstetric suboptimality was mediated by confounders such as birth order (Lord, Mulloy, Wendelboe, & Schopler, 1991).

In two recent meta-analyses, Gardener and colleagues included forty studies, and found an association between ASD and several obstetric factors including: parental age at birth, maternal prenatal medication use, gestational diabetes, uterine bleeding, birth order, abnormal presentation, umbilical-cord complications, foetal distress, birth

injury or trauma, multiple birth, maternal haemorrhage, low birth weight, small for gestational age, congenital malformation, low 5-minute Apgar score, feeding difficulties, meconium aspiration, neonatal anaemia, ABO or Rh incompatibility, and hyperbilirubinemia (Gardener, Spiegelman, & Buka, 2009, 2011). Other authors, however, reported different conclusions. Kim et al in a recent umbrella review of meta-analyses found an increased risk of ASD with maternal hypertension (Kim et al., 2019), reporting inconsistent results for other obstetrics factors (Kim et al., 2019) . Similarly, Carlsson et al reported in a systematic review of twin and siblings studies that low birth weight (BW) was the only risk factor with consistent results across studies (Carlsson, Molander, Taylor, Jonsson, & Bölte, 2020).

Twin studies have been used to study the association of obstetric factors and ASD because they allow exploration of the environmental versus genetic influences on a variable. Families share factors that are unmeasured in classical observational studies, which is a strong limitation when examining causal relations between environmental factors and psychopathology. Several studies have shown that the association of some environmental factors and psychiatric disorders became attenuated or nonsignificant when a family-based study design was used (Curran et al., 2015; Iliadou et al., 2010; Rai et al., 2017).

Here, we aimed to explore the association of obstetric complications (OC) and ASD using different study methods. First, we use data from the same population based twin study as Ronald et al, but we considered ASD best estimate diagnosis established through in person assessment at age 12-15 years, to explore obstetric factors associated with autism. Secondly, we aimed to examine the published results from other family studies reporting obstetrical factors and ASD. Thus, we did a meta-analysis for studies including a sibling comparison that included obstetric factors and autism.

METHODS

Twin study

Participants

The participants were recruited from the Twins Early Development Study (TEDS). TEDS is a longitudinal population-based case-control twin study, described in detail elsewhere (Rimfeld et al., 2019). The families of twins born between January 1994 and December 1996 in England and Wales were identified from birth records. They were contacted by the Office of National Statistics (ONS) and invited to enrol in the current study (pre-registered: <https://osf.io/cjrz8>).

ASD and co-twin sample

Between 2004 and 2007, families with at least one twin with possible ASD were identified if a) they reported an existing ASD diagnosis or b) at age 8, one or both twins scored 15 or above on the Childhood Autism Spectrum Test (CAST) (Williams et al., 2005).

A total of 412 families were identified as having at least one twin with an ASD diagnosis/high autistic traits, and were invited to participate in the Social Relationship Study (SRS). During the first stage, they completed the Developmental and Wellbeing Assessment (DAWBA) (Goodman, Ford, Richards, Gatward, & Meltzer, 2000). Families where at least one child met the criteria for ASD were invited to take part in Stage 2 of the study, at which point in person gold-standard autism diagnostic assessments (ADI-R and ADOS-G, see below) were administered when the twins were aged 12-15 years.

Control sample

80 families who scored low on CAST (below 12) at age eight from the original TEDS study were invited to take part in the study as control families. They were matched to case twins on gender, zygosity, age and socio-economic status (SES)

Obstetric measures

Parents of case and control twins completed an interview regarding obstetric information using the Obstetric Enquiry Schedule (OES) (Bolton et al., 1994). The OES is an 82-item interview that asks about pre, peri and neonatal variables. The questionnaire was based on the Gillberg Optimality Scale (Gillberg & Gillberg, 1983) and the Rochester Obstetric Scale (Zax, Sameroff, & Babigian, 1977). To obtain a “sub-optimal score” (SO), some of the variables were scored according to Gillberg and Gillberg (*See appendix for a full list of items*). A score of 0 was given to optimal conditions and a score of 1 to those variables outside of the optimal range. The final score was obtained by adding up all the items. The scale was also divided into pre, peri and neonatal subscales. High scores in the SO scale indicate a greater number of OC. A modified scale including factors unique to each child was created in order to run within-twins analysis (Appendix, table S2).

Analysis of missing data was carried out using Little’s Missing Completely at Random (MCAR) test (Little, 1988). The test showed that less than 5% of data was missing across all the variables. Therefore, we decided to exclude the missing data.

To assess the validity of the OES scale completed when the twins were aged 12-15 years, the data were compared to the information obtained through questionnaires given to parents as part of the first contact from TEDS, when the twins were 18 months old. The scores in each scale were added, obtaining two continuous scales. The scales included gestational age (<37 or >37 weeks), vaginal bleeding, maternal diabetes mellitus (DM) during pregnancy, maternal high blood pressure during pregnancy, induction of labour, caesarean section, rupture of membranes early during pregnancy, and use of anaesthesia during labour. The agreement between the two scales was assessed using intra-class correlation (ICC) (Ranganathan, Pramesh, & Aggarwal, 2017), using absolute-agreement, and 2-way mixed-effects model. The value of ICC was 0.84 (95% CI 0.80-0.87, $p < 0.001$), which is indicative of good reliability (Koo & Li, 2016).

ASD assessment

Childhood Autism Spectrum Test (CAST)

CAST is a 31-item questionnaire completed by the carer. It assesses autistic behaviours and has been shown to have good accuracy as a screening test. Compared to consensus diagnosis for ASD, CAST has a sensitivity of 100%, specificity of 97%, and a positive predictive value of 50% (Williams et al., 2005). All the questions are answered yes or no, and the cut-off for possible ASD is at 15 points (Williams et al., 2005). It was completed by the parents of the twins at the age of eight years.

Developmental and Wellbeing Assessment (DAWBA)

The DAWBA (Goodman et al., 2000) is a structured interview that generates International Statistical Classification of Diseases and Related Health Problems 10 (ICD-10) and DSM-IV psychiatric diagnoses. It was reported to have a sensitivity of 88%, and specificity of 85% when compared to ASD diagnosis obtained through ADI-R and ADOS (McEwen et al., 2016). Parents were interviewed by phone and the ASD module of DAWBA was used. This included 14 questions about repetitive and restrictive behaviours, 15 questions about social difficulties, and three questions about language development. The provisional diagnosis of ASD was given if they met criteria for ICD-10 and DSM-IV.

Autism Diagnostic Interview–Revised (ADI-R)

The ADI-R is a semi-structured interview for caregivers, which explores developmental history and focuses on the following domains: language and communication, reciprocal social interactions, and restricted, repetitive, and stereotyped behaviours and interests (Lord, Rutter, & Le Couteur, 1994).

Autism Diagnostic Observations Schedule (ADOS)

The ADOS-G is a semi-structured assessment used for the observation and evaluation of communication, social interaction, play, and restricted and repetitive behaviours. The ADOS-G assessment module is chosen based on both the age and language level of the individual to be assessed. (Lord et al., 2012).

We used the calibrated severity scale for the ADOS to quantify ASD severity. The use of this scale allowed us to compare children's scores regardless of the module used (Gotham, Pickles, & Lord, 2009).

Diagnostic consensus

Best estimate research diagnoses were assigned after reviewing all the available information: DAWBA, ADI-R, ADOS and clinical information. When there were inconsistencies in the diagnosis across instruments, two investigators assigned the diagnosis through consensus (PB & EC). Initially we created three categories: no autism, autism, and broad spectrum. Later, in keeping with DSM-5, we combined broad spectrum and autistic disorder to form an ASD category.

Cognitive functioning of twins

Intellectual and cognitive functioning was assessed when twins were approximately 13 years of age using the Wechsler Abbreviated Scale of Intelligence (WASI). A total of 30 children did not have the verbal or language skills to complete the assessment. For the purpose of the current study, a score of 50 was given to these children to enable the calculation of intelligence quotient (IQ) differences within twin pairs. Only the WASI composite score was used in the current investigation.

Zygoty

Zygoty was assigned when children were 18 months of age at first TEDS contact. Assignment was based on polymorphic DNA markers using DNA that was extracted from cheek swabs.

Socio Economic Status

Socio economic status (SES) was gathered at TEDS first contact, based on five measures: mother's highest qualification, father's highest qualification, mother's social class (from unskilled occupation to professional), father's social class, and mother's age when the first child was born. The five measures were combined to create a single composite score by standardizing the variables and taking the average SES score.

Statistical Analysis

First, using all the twin pairs included in the SRS, demographic and sub-optimality differences between twin pairs from case versus control families were explored using non-parametric tests.

Then, to account for twin relatedness, a logistic regression with random effects was used, having individuals in level 1 and twin pairs in level 2. The categories ASD, no autism and control were used as outcomes. The participants in the "no autism" category were the co-twins of participants in the category of ASD who scored below cut-off on the ASD assessment. Maternal age, sex, SES and IQ were included in the fixed effect model as confounders. The Family identity number (ID), to represent relatedness between twins, was included in the random effect model.

Linear mixed models were used to assess the association between obstetric and birth complications and autistic traits, measured by CAST. Maternal age, sex, IQ and SES were included in the model as confounders.

In the next stage, we explored if, within twin pairs where at least one had a diagnosis of ASD, prenatal and neonatal factors were associated with the severity of symptoms. We used linear mixed models, where the "severity scale" of ADOS was used as the outcome.

Confounders included maternal age, sex, IQ and SES and were selected based on previous literature (Delobel-Ayoub et al., 2015; Langridge et al., 2013; Sandin et al., 2012; Werling & Geschwind, 2013).

Values were considered significant if $p < .05$. All values were 2-sided, unless specified. The analyses were performed using SPSS Statistics version 26.

Meta-analysis

A meta-analysis of published cohort and case-control sibling studies was carried out. The meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The PRISMA checklist can be found in the appendix (Table S5).

Search strategy and study criteria

We conducted a review in the databases Medline, PsycINFO and EMBASE from inception to July 1, 2021. The search was limited to studies published in English with human participants. The full electronic search strategy can be found in the appendix. First, the articles were screened based on the title and abstract. From the abstracts selected, the full texts were screened again to confirm their eligibility to be included. The inclusion criteria included (1) observational studies (both case-control and cohorts studies), (2) a sample of subjects with a diagnosis of ASD (2) a comparison group of their unaffected siblings, (3) pre-, peri- or neonatal factors, and (4) enough data to allow the calculation of effect size. Exclusion criteria included reviews, conference abstracts and studies with not enough data to do the analyses. When two studies presented overlapping populations, the study with the largest sample or the most similar factor to other studies was included in the analysis.

Data extraction and analysis

From the studies selected, data was extracted and included in an Excel spreadsheet. Variables extracted were: first author, publication year, country, type of study, sample size, ASD diagnostic instrument and risk factors included. The risk of bias was rated according to the Newcastle-Ottawa Scale (NOS) (Wells et al., 2019).

We examined the association between each of the factors selected and autism. We used preferably raw data to calculate odds ratio (OR) or risk ratio (RR). If binary data was not available, we extracted OR, RR or hazard ratio (HR) and 95% CI. As the prevalence of ASD is low (<10%), we considered the HR and RR equivalent. If the variable was continuous, the mean difference was used as the effect size.

We did a meta-analysis of each factor identified in at least 3 different samples. To account for the heterogeneity of the studies, we used random effects. We calculated pooled effect size and 95% CI.

Q and I^2 statistics were used to quantify between-studies heterogeneity. For the Q statistic, it was considered that a p-value <.05 was indicative of significant heterogeneity. For the I^2 statistic, values of 25%, 50% and >75% were suggestive of low, moderate and high heterogeneity, respectively (Higgins, Thompson, Deeks, & Altman, 2003). Publication bias was assessed by visualizing the funnel plot, as well as using Egger tests (Egger, Smith, & Minder, 1997) and Duval and Tweedie's nonparametric trim and fill method (Duval & Tweedie, 2000).

The analyses were performed using Stata, version 17.0 (StataCorp).

RESULTS

Twin study

The demographics of case and control families are shown in Table 1. Case and control families were similar in terms of twin sex, order of birth within the pair and SES, but differed in zygosity, maternal age at birth and cognitive functioning. CAST scores at age eight were, as expected, significantly higher among case than control pairs.

The results for the sub-optimality scale were significantly higher in the case families, both for the total score as well as for the subscales of prenatal, perinatal and neonatal factors.

Insert here Table 1

The generalized mixed model analyses showed statistically significant differences between the control and autistic children on the total sub-optimality score (control mean 6.93, sd 2.98; ASD mean 8.68, sd 3.37) as well as the neonatal subscale (control mean 1.11, sd 1.40; ASD mean 1.99, sd 1.11). The estimate for prenatal subscale (control mean 2.32, sd 1.56; ASD mean 2.68, sd 1.46) did not remain significant after adjusting for IQ. Similarly, statistically significant differences were identified between unaffected co-twins of ASD probands and controls on the total scale (co-twin mean 8.40, sd 3.09), prenatal (co-twin mean 2.74, sd 1.51), and neonatal subscales (co-twin mean 1.88, sd 2.00). No statistically significant differences were observed between autistic children and their unaffected co-twins on any of the sub-scales (see Table 2).

Insert here table 2

The linear mixed model showed a significant associations between the CAST scores and the total and all subscales except perinatal (see Appendix, table S4). No significant association was observed between OC and higher scores on the severity scale of the ADOS ($p=.31$).

Meta-analysis

1731 papers were identified in the electronic search, with an additional paper from other sources (Figure 1). In total, 24 papers were included in the meta-analysis (13 cohort, 10 case-control and 1 cross-sectional study). The included articles were published between 1980 and 2021 and all of them included a categorical diagnosis of ASD. The sample sizes ranged from 30 to 993,301. The details of the studies are included in the Appendix (Table S6).

Insert here Figure 1

Ten risk factors were included in the meta-analysis (see Table 3). Maternal hypertension (OR 1.35, 95% CI 1.23, 1.48), uterine bleeding (OR 1.20, 95% CI 1.01, 1.42 and exposure to antibiotic during pregnancy (OR 1.10, 95% CI 1.00, 1.22) were statistically

significant risk factors in autistic participants in comparison to their unaffected siblings. We detected potential publication bias for the three significant risk factors. However, the results after including studies imputed by the trim-and-fill method were very similar to the initial results (OR, 1.36 [95% CI 1.24, 1.45], 1.12 [95% CI 0.99, 1.23] and 1.16, [95% CI 1.07, 1.26] respectively). Moderate heterogeneity (I² 50%-75%) was found for the factors maternal age and C-section.

Insert here Table 3

DISCUSSION

This investigation evaluated the influence of OC on ASD using two different study methods and controlling for familial confounding. First, we used a population-based case control design using a British twin sample to examine the association between sub-optimal conditions during pregnancy and the risk of ASD. This showed that OC do not differentiate autistic young people and their unaffected twins. Secondly, we pooled together the results of individual factors from previous published sibling studies using meta-analysis and we observed that family studies showed little influence of birth complications on autism. Our findings stressed the importance of familial confounding when studying environmental factors and their role in NDD.

Due to the inconsistent results found in previous studies when examining specific factors, we summed different factors to create a scale of “obstetric sub-optimality” and examined its association with ASD. The results found that children later diagnosed with ASD presented a higher number of complications compared to a control group matched in gender, zygosity, age and SES. However, they did not differ significantly in obstetric suboptimality from their non-autistic co-twins, who also showed significantly worse scores than controls. These results are consistent with those reported by other authors (Bolton et al., 1997; Brimacombe, Ming, & Lamendola, 2007; Dodds et al., 2011; Gillberg & Gillberg, 1983; Juul-Dam, Townsend, & Courchesne, 2017; Lyall, Pauls, Spiegelman, Ascherio, & Santangelo, 2012), while some other authors have found that this association did not remain significant after adjusting for confounders, such as birth order (Lord et al., 1991; Piven et al., 1993). In the present data the association between sub-optimality and ASD family status remained significant in analyses adjusted for sex, maternal age and IQ.

When examining the subscales, only the sum of the neonatal items differentiated autistic young people from controls but did not differentiate autistic twins from their

unaffected co-twins. In unadjusted analysis, prenatal factors also differed statistically between the two groups, but adjusting for IQ rendered this association non-significant. Whereas these results are in line with those reported by Stein et al (Stein, Weizman, Ring, & Barak, 2006), some other authors have reported a higher number of prenatal complications in autistic children compared to controls after controlling for IQ (Bryson, Smith, & Eastwood, 1988; Lord et al., 1991). The lack of consistency might be due to differences in sample selection. For instance, the studies that found a significant difference in prenatal factors included participants that met DSM-III-R criteria for Autistic Disorder. By contrast, the criteria have changed over the last years and the diagnosis has become broader, which might influence the differences found in our study and those previously reported.

We also found that autistic subjects do not present significantly more OC than their unaffected co-twins. One explanation for this could be that some of the co-twins identified as “no autism” exhibited milder symptoms of the spectrum that were not detected in our study. However, we used a two-step systematic screening method, including in person gold-standard diagnostic instruments, which aimed to identify ASD exhaustively. As the association observed did not remain significant when using familial comparison, the OC observed in autistic children compared to controls might be the result of confounding of shared factors related to genetics, rather than a causal association. We also observed a significantly higher number of complications among unaffected co-twins compared to controls, which would support the hypothesis that obstetric adversities are the result of some shared familial risk factors (Bolton et al., 1997). The use of twin data allows us to adjust for factors, genetic or environmental, that are currently unknown and might be acting as confounders. This would explain the discrepancy between results from non-twin studies and results from our study. Our results are in line with recent publications examining neurodevelopmental disorders and risk factors, where the association between risk factors and neurodevelopmental

disorders disappears when comparing within families. For instance, Curran et al found an association between C-Section and ASD. However, the association did not remain significant when adjusted for siblings (Curran et al., 2015). Likewise, Obel et al found no association between maternal smoking and ADHD in a siblings study (Obel, Zhu, Olsen, & Rutter, 2016). Thus, causal inferences in epidemiological studies should be interpreted with caution, as measured or unmeasured genetic and shared environmental factors are an unaddressed source of bias in these studies.

Due to this potential source of bias, we then aimed to explore family studies that examined risk factors and ASD. Due to the small number of twin studies, all the papers included in the meta-analysis were sibling studies. This is, to our knowledge, the first meta-analysis combining different obstetric risk factors and autism in sibling studies.

In the meta-analysis, we found that maternal hypertension, uterine bleeding and exposure to antibiotic during pregnancy are risk factors for ASD. However, the results showed that the influence of each risk factor is relatively small. This might be due to the limited number of studies included in each category as well as the small samples sizes of some studies. In a recent umbrella review of meta-analysis of singletons Kim et al categorised the evidence according to criteria of the meta-analyses included, such as p values or sample size. The categories were 'convincing', 'highly suggestive', 'suggestive', 'weak' and 'not significant'. They reported maternal hypertension as showing "convincing evidence" of an increased risk for ASD (Kim et al., 2019). However, a review of studies using twin and sibling comparison, only found an association between increased risk of ASD and low BW (Carlsson et al., 2020).

These outcomes show that, regardless of the study design and despite the current broader criteria for autism, the results of any kind of association between specific obstetric factors and ASD remain inconsistent over the years. The findings from both our studies support the hypothesis that the modulation of OC on the risk of ASD might be

due to other non-measurable factors that are not currently included in studies. Thus, the role of specific obstetric factors does not appear to be interpretable at an individual level.

Limitations and strengths

The use of a population-based twin sample enabled us to capture cases with subtle difficulties that may not have been diagnosed at early ages, and use of in person assessments allowed us to rule out those cases that may have scored high in some screening tests for reasons other than ASD. Additionally, the twin study design allowed us to account for potential genetic, environmental and epigenetic confounders. We also used the best estimate diagnosis to ensure the inclusion of the complete genetic picture. This is, to our best knowledge, the largest twin study examining OC and autism that used a robust diagnostic assessment.

However, the study has some limitations that should be noted. Firstly, the data were obtained through an interview with mothers which relies on maternal recall of pregnancy-related information. Parental recall may be biased by later child outcome. However, we compared our data with the information obtained when the twins were 18 months old and did not have yet a diagnosis of ASD. We showed a good correspondence between the information obtained more than a decade apart, which suggests relatively low risk of recall bias. Secondly, our sample was relatively small. As a result, the results require confirmation from larger samples. Lastly, although twin studies offer a unique possibility of testing causality in cases where randomization is not possible, there are potential limitations with these designs. For example, it has been reported that twins are more susceptible to certain OC, such as being born premature or the twin-to-twin transfusion syndrome (Ronald & Hoekstra, 2011). However, the evidence does not suggest that ASD or ASD traits are more common in twin pairs compared to singletons (Curran et al., 2011).

In regard to the meta-analysis, the number of studies included in each category was small (from 3 to 6 studies). Large cohort studies with sibling comparisons are limited, and therefore further meta-analysis with larger studies are needed.

Conclusions

This investigation including two different methods studies confirms that (1) OC do not distinguish between ASD twins and their unaffected co-twins and (2) the modulation of OC on autism risk is small. Our results suggest a shared familial liability for both OC and autism, rather than a causal association.

Conflicts of interest statement: No conflicts declared.

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Key points

- Research has shown ASD is influenced by environmental factors, although it is unclear which factors play a role.
- In meta-analyses of sibling studies we identified maternal hypertension, uterine bleeding and exposure to antibiotic as factors that increase risk of ASD, although the influence is small.
- In our study we observed that children with ASD do not present more obstetrical complications than their unaffected twins.
- Therefore, we concluded that the increased number of obstetric complications in autistic children is the result of shared familial liability for obstetric complications and ASD.

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TABLES AND FIGURES

Table 1: Demographics and Obstetric Suboptimality results for case (i.e. including at least one ASD twin) and control families/twin pairs: mean, (s.d.) unless otherwise specified.

	Control pairs	Case pairs	p-value	Cohen's d
N	124	230		
Sex			.59	
Male N (%)	90 (72.6%)	161 (70%)		
Female N (%)	34 (28.4%)	69 (30%)		
MZ N (%)	40 (32.3%)	50 (21.74%)	.03*	
DZ N (%)	84 (67.7%)	180 (78.26%)		
Birth order			1.00	
1 (%)	62 (50%)	115 (50%)		
2 (%)	62 (50%)	115 (50%)		
Maternal age (yrs)	32.82 (3.89)	31.20 (4.68)	.003*	.33
SES	0.24 (0.58)	0.15 (0.76)	.30	.13
IQ	105.11 (15.17)	94.72 (23.1)	<.001*	.47
CAST at 8	4.30 (2.52)	12.12 (8.34)	<.001*	.29
Sub-optimality score				
Total	6.26 (2.85)	7.83 (3.12)	<.001*	.49
Prenatal	2.36 (1.58)	2.71 (1.48)	.01*	.27
Perinatal	2.78 (1.15)	3.13 (1.15)	.01*	.26
Neonatal	1.11 (1.40)	1.99 (2.04)	<.001*	.40

MZ: Monozygotic; DZ: Dizygotic; SES: Socio economic status; IQ: intelligence quotient; SO: Sub-optimality scale; CAST: Childhood Autism Spectrum Test

Table 2: Results from the generalized linear mixed models, using ASD diagnosis as outcome. Scores from SO, SO prenatal, SO perinatal and SO neonatal were used as fixed effects. Family ID was used as random effect. Adjusted for gender, maternal age at birth, SES and IQ.

Sub- optimality scale	Unaffected co-twins vs								
	ASD vs controls			controls			ASD vs unaffected co-twins		
	β (95% CI)	t	p	β (95% CI)	T	p	β (95% CI)	t	p
Total	1.25 (1.11-1.40)	3.81	<.001*	1.20 (1.07-1.36)	3.00	.003*	0.96 (0.85-1.09)	- .60	.55
Prenatal	1.27 (0.98-1.64)	1.80	.073	1.34 (1.04-1.74)	2.23	.03*	1.06 (0.81-1.40)	.44	.66
Perinatal	1.15 (0.90 – 1.47)	1.12	.25	1.12 (0.87-1.46)	0.89	.37	1.00 (0.76-1.32)	.02	.99
Neonatal	1.35 (1.08-1.67)	2.68	.008*	1.32 (1.06-1.65)	2.46	.01	0.96 (0.78-1.17)	- .44	.66

SO: Sub-optimality scale. SES: Socio economic status; IQ: intelligence quotient;

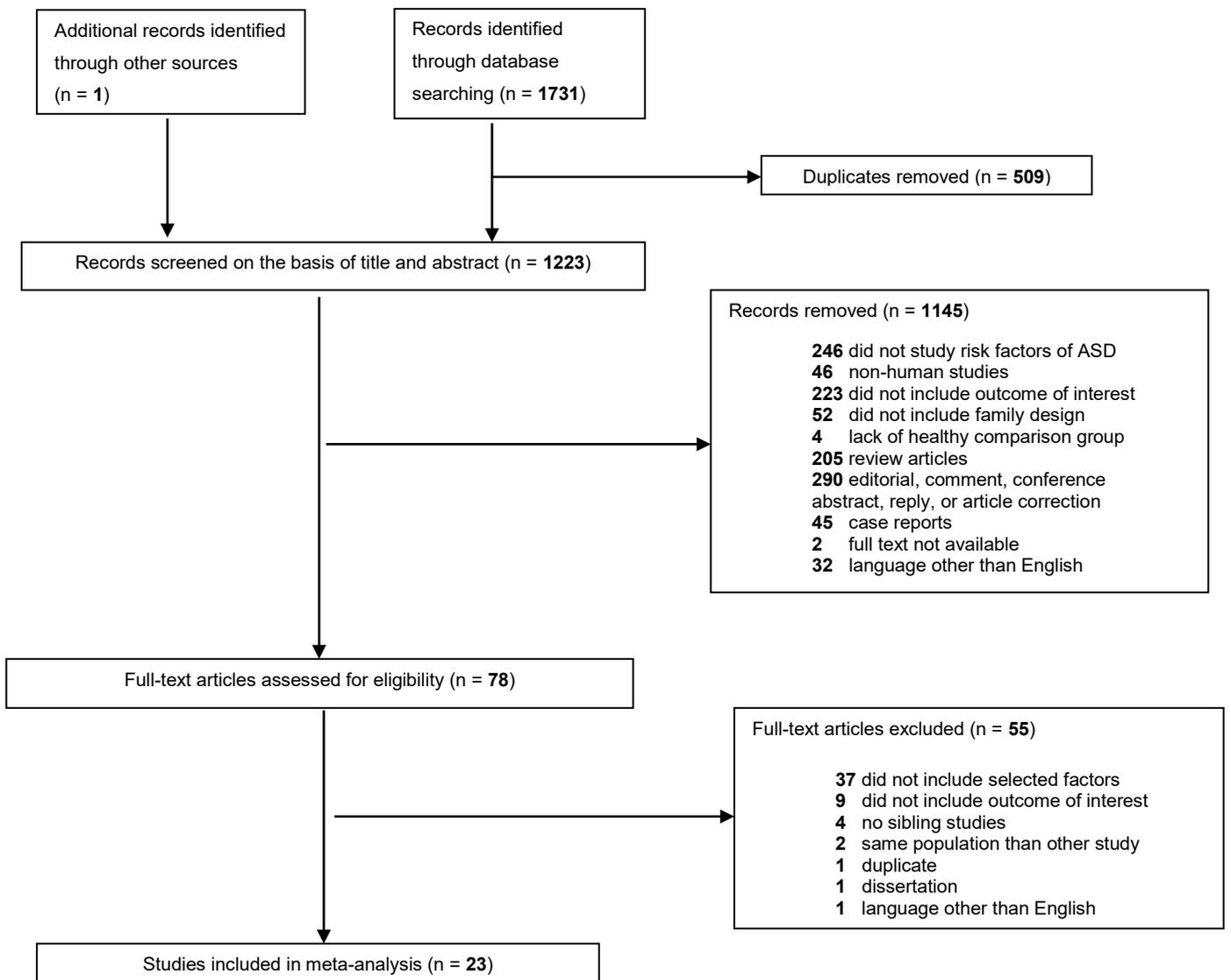


Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Flow Diagram

Table 3: Meta-analytic association between obstetric factors and ASD. Random effects were used for all the analyses.

Factor	Studies (n)	Sample	Effect measure	p value	Heterogeneity		Publication bias		
			ES (95% CI)		I (%)	p value	Trim and fill imputed studies	Trim and fill adjusted ES (95% CI)	Egger p value
Maternal age	3	1299	-.124 (-.306, .057)	.179	54.9%	0.109	0	N/A	0.439
Gestational age (<37 weeks)	4	325	1.209 (.872, 1.676)	.256	<.001	.876	0	N/A	.709
Low birth weight (<2500g)	4	671	1.237 (.848 - 1.805)	.114	35.5%	.199	0	N/A	.933
High blood pressure during pregnancy	3	7.021	1.350 (1.234 , 1.478)	<.001*	<.001	.415	2	1.360 (1.244, 1.486)	.281
Uterine bleeding	6	2.134 999281	1.198 (1.011-1.421)	.018*	22.8%	.263	2	1.120 (.990 - 1.267)	.397
Maternal smoking	5		1.076 (.957, 1.211)	.102	17.9%	.301	0	N/A	.769
Maternal antibiotic use	3	747.917	1.105 (1.002, 1.219)	.046*	<.001	.656	2	1.160 (1.067, 1.261)	.971
Maternal antidepressant use	4	33.483	1.120 (.778, 1.611)	.921	41.2%	.164	2	.884 (.587, 1.331)	.193
C-Section (planned/unplanned)	3	525	1.159 (.695, 1.935)	.863	64.6%	.059	0	N/A	.682
Unplanned C-Section	4	809.810	.892 (.739, 1.077)	.070	74.9%	.008	1	.943 (0.773, 1.149)	.507

ES: Effect size (OR, RR).